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Cellular and Molecular Mechanisms Governing Functional Recovery of Dementia Mice after Neuronal cell Transplantation

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Abstract

Massive degeneration of the basal forebrain cholinergic cells is one of the major histopathological changes of Alzheimer's disease (AD). The degeneration leads to Acetylcholine (ACh) deficits in the cortex and hippocampus. AD disease severity was negatively correlated with ACh expression. We conducted neuronal transplantation with cells derived from Human Induced Pluripotent Stem (hiPS) cells into the bilateral hippocampi of human amyloid precursor protein transgenic AD mice. The transplantation significantly improved cognitive dysfunction in the mice.

Transplanted neurons differentiated further in the host cortex into human Choline Acetyl Transferase (ChAT)+ cholinergic neurons. In the hippocampus, the grafted cells preferentially differentiated into human vesicular GABA transporter (VGAT)+ cells. We suggest that transplanted neurons may compensate for neurotransmitter loss associated with AD lesions.

In this review, we summarize current topics of neurotransmitter system perturbation of AD pathology. We would like to emphasize the importance of GABA/GABA receptor (GABAR) circuits as well as ACh/ACh receptor (AChR) pathways in the hippocampus reconstituted by the transplantation.

Keywords

Human induced pluripotent stem cells; GABA; Choline acetyl transferase;Vesicular GABA transporter; Interneurons; Hippocampus

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Introduction

Human Induced Pluripotent Stem (hiPS) cell transplantation therapy for a degenerative eye disease has been reported [1]. Thereafter, the treatment has become to receive much attention for its potential to rescue the impaired cellular functions of various neurological disorders, including cognitive dysfunctions in patients with Alzheimer's disease (AD).

We previously transplanted hiPS cell-derived neuronal cells into the hippocampus in AD model mice [2,3]. The cell transplantation significantly improved cognitive dysfunction in the mice. The transplanted neurons differentiated into cholinergic and GABAergic neurons predominantly in the cerebral cortex and hippocampus, respectively. The characteristic human neuron distribution in the mouse brain may be partly due to the microenvironments of the AD lesions, including secretion of chemokines and growth factors, leading to neuronal cell activation/migration.

It is well known that cholinergic neurons which secrete acetylcholine (ACh) play important roles in learning and memory functions. The activity of cholinergic neurons and their Ach production are down-regulated in patients with AD [4,5], and down-modulation of alpha7 nicotinic ACh receptor (alpha7 nAChR) has been reported as one of the hallmarks of AD [6].

On the contrary, previous studies of GABA expression in AD were inconsistent. Several researchers have reported that the hippocampal GABAergic system was resistant to the AD pathology to some extent [7].

We here focus on neurotransmitter secreting neurons derived from hiPS cells and respective receptor-expressing neurons in the hippocampus of grafted AD model mice. It is possible that the neurotransmitters secreted by the grafts are involved in the functional and morphological improvements of mice after the transplantation.

Ach and GABA in the AD hippocampal formation

Basal forebrain cholinergic cells provide the cholinergic projections to the cerebral cortex and hippocampus. Progress has been made in research into the neuropathology

of cells and nucleus basalis of Mynert in patients with AD. The cell complex is suggested to be divided into four groups, termed Ch1-Ch4, based on anatomical features, such as nucleus size, neural density, acetylcholinesterase immunoreactivity, and the innervation area [8]. Ch1/Ch2 (medial septal nucleus/vertical limb of the diagonal band nucleus) projects to the hippocampus, whereas Ch3 (horizontal limb of the diagonal band nucleus) projects to olfactory bulb.

The Ch4 group is the largest out of the four cholinergic neuron groups and corresponds well with large parts of nucleus basalis of Mynert. The most AD affected region was the posterior part of Ch4 group, and the innervated superior temporal and temporal polar neural loss correlated well with memory loss and language impairment of AD [8,9]. In contrast, AD neurons of Ch1/Ch2, where the projection lead to the hippocampus, exhibited only modest degeneration compared with age-matched control individuals [10,11].

The Ch1/Ch2 complex, which mainly innervates the CA2/CA3 regions of the hippocampus [12], plays an important role in learning and memory [13]. Alpha7 nAChRs are expressed densely in GABAergic hippocampal interneurons [14], whose signals are modulated by cholinergic axons [15]. The most distinctive AD associated neural loss was observed in the CA1 region of the hippocampus [16].

One of the major pathological features of AD is the substantial reduction of cortical cholinergic markers, such as ChAT and acetylcholinesterase [17]. Distinct nAChR subunit loss in the cortex and hippocampus was also reported to be related to AD pathology [18].

The findings of AD-related alterations in GABAergic neurotransmission are inconsistent in both humans and animal models [19]. In the early stages of AD, the hippocampal GABAergic system was suggested to be relatively resistant to AD pathology compared with glutamatergic neurons [7].

It was recently reported that down-regulation of Glutamic Acid Decarboxylase (GAD)67, an enzyme that synthesizes GABA, was more severe than previously thought in AD patients [20,7]. Researchers found that distinct parts of GABAR subunits reduced in their expression in the AD hippocampus [7]. Additionally, postmortem studies suggested that GABAAR expression was decreased in cortical areas of AD patients [21,22]. Loss of functional GABAARs in the AD brain was also observed [23].

These data suggest that both cholinergic and GABAergic systems are substantially affected, at least in the later stages of AD.

Neuron transplantation in AD models

Researchers have transplanted various stem/progenitor cells, such as mouse [24-31] and human [32-36] neural stem/progenitor cells, mouse [37-41] and human [42,43] mesenchymal stem/progenitor cells, Embryonic Stem (ES) cell-derived neural cells [44,45], iPS cell-derived neuronal [2,44,3] and myeloid [46] cells, and umbilical cord stem cells [47] into AD models. The cell transplantations significantly improved cognitive functions.

In histopathological analyses, the transplanted cells promoted the formation of synapses [24-27,33-35,29] and decreased amyloid-beta burden [42,24,44,25,47,27,34,39,40,29,41].

The cells were transplanted into the hippocampus and/or ventricle(s) of model mice mainly with the use of needle puncture. The grafted neural cells showed wide variation in their distribution, probably due to differences in the characteristics of the grafts, the transplantation procedures, and the observation periods. When the grafts were injected into ventricles, grafted cells were distributed widely around the ventricles [34] and whole brain [32,37], approximately 1 - 2 months after the transplantation.

In other experiments, cells transplanted into the hippocampus remained in close proximity to the grafted sites for 2 – 9 months [44,29,36] and cell numbers gradually decreased [29,36,43].

The transplanted neural stem/progenitor cells tended to migrate further than mesenchymal stem cells, from the hippocampus to the cortex [24-26,33,3,31]. After the migration, the neural cells differentiated into mature neurons [31-34,2,25,3,26], astrocytes [24-26,33,34,31], and oligodendrocytes [24,34,31].

Generation of basal forebrain cholinergic neural cells from human ES cells has been reported[45]. Yue et al. transplanted these neurons into the basal forebrain of AD model mice, and cognitive dysfunction was significantly improved. The neural progenitor cells migrated along cholinergic projection track from the nucleus basalis of Mynert towards the cortex and hippocampus. The synaptic formation between host neurons and grafted cells suggested that ES cell-derived neurons were successfully incorporated into the endogenous cholinergic projection system.

Of note is that the grafted mouse neural stem cells differentiated into cholinergic neurons and exhibited similar positive effects on ACh concentrations in the brain and cognitive functions in AD model mice [26]. ChAT overexpressing neural stem cells [32] significantly shortened the escape latency of the Morris water maze test after transplantation, suggesting that oversupply of cholinergic neurotransmitters improved cognitive function of AD models.

Collectively, the transplantation into basal forebrain restored the cholinergic projection system and cognitive function in AD rodent models.

Functional study of learning capability and histological analyses of mice grafted with hiPS cell-derived neuronal stem/progenitors

PDAPP transgenic mice, which overexpress mutated human APP (APPV717F)[48], display progressive synaptic loss [49], reduction in the size of the hippocampus, and spatial memory dysfunction starting from a few months of age [50,51]. Furthermore, PDAPP mice have been shown to have reduced levels of hippocampal Ach[52].

Mice were left on the platform for 30 seconds before the next trial was started. For all tested mice, we calculated the average value of the latency from the four trials performed on each trial day, and we showed this value as the mean escape latency.

We found that neural cell transplantation improved cognitive functions (as shorter mean escape latency) of dementia mice (Figure 1A). Figure 1B depicted tracing of the mouse swimming path. Indeed, total swimming time until reaching to the platform of the grafted mice was clearly shortened.

Histological analysis disclosed that ChAT+ neurons distributed throughout the overlying cerebral cortex around the injection site (Figure 2). ChAT+ neurons composed a quarter of the nucleated cells, of which were half human neurons, and the remaining half were mouse neurons. In the cortex of the grafted mice, half of the nucleated cells were alpha7 nAChR+ neurons. It was surprising that the distribution of cholinergic neurons and GABAergic neurons was clearly and consistently different from each other after the transplantation. We suggested that cortex-locating grafts may compensate for the depletion of Ach in the cortex, which was caused by the basal forebrain Ch4 projection loss [3].

In the hippocampus, ChAT+ neurons were located around the injection site in the DG. In the hippocampus, one third of the nucleated cells were alpha7 nAChR+ neurons. A substantial numbers of mouse ChAT+ neurons and mouse alpha7 nAChR-expressing cells were observed in the grafted mouse hippocampus. Thus, it was possible that hiPS cell-derived neurons altered the differentiation of mouse neural stem/progenitor cells, increasing ChAT+ neurons and alpha7 nAChR expressing cells in the grafted mice. These ChAT+ neurons emerged after neuronal cell transplantation in both the cortex and hippocampus, and may contribute to the functional recovery of PDAPP mice. However, detailed analyses of the grafted mice with regard to their ChAT+ and receptor expressing neurons, especially neuronal circuits reconstituted by the grafts, are yet to be performed.

As mentioned previously, AD findings in the human GABAergic system were inconsistent, but GABAergic interneuron loss was obvious in several AD models [53]. Impaired GABA functions were observed in PDAPP mice [54], tau protein transgenic mice [55], and apolipoprotein (apo) E4 knock-in/APP mice [56]. These mice exhibited defective hippocampal functions including GABAergic neuronal loss and/or dysfunction and memory deficits.

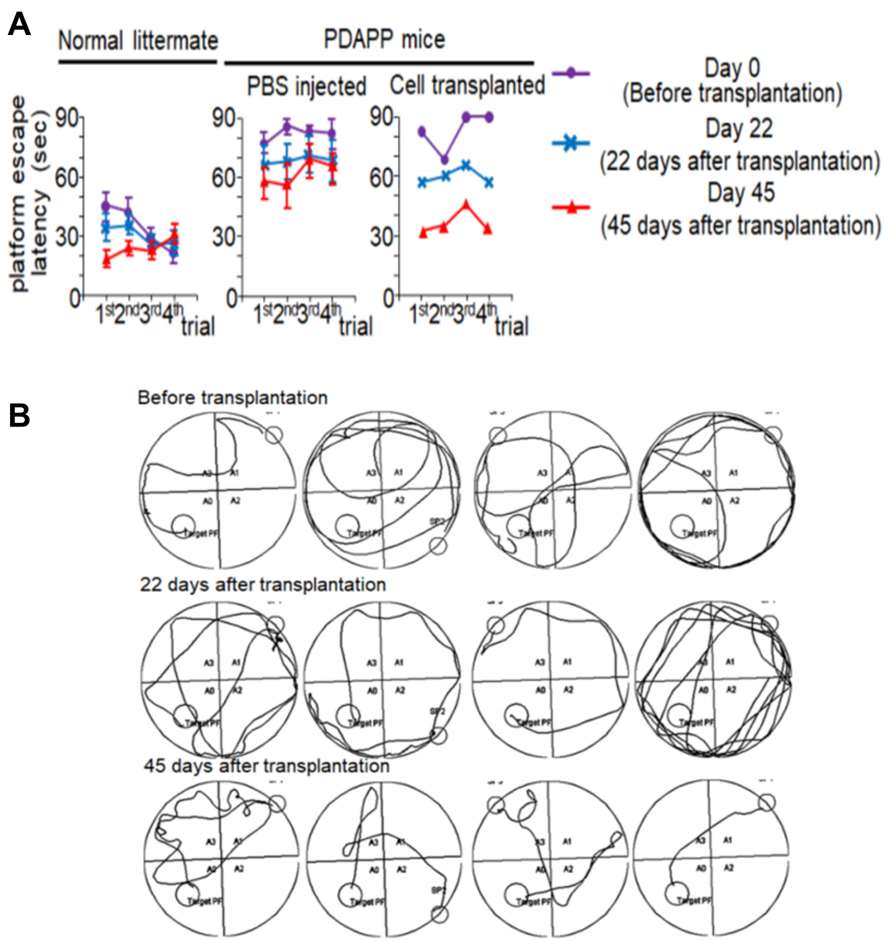

Figure 1: Transplantation of the neural stem/progenitor cells restored spatial memory learning in dementia model mice.

A. Hidden test was conducted for 4 consecutive days after visible test. Twenty-two days after neural cell transplantation, the grafted mouse showed shortening of mean platform escape latency. The improvement has become more evident 45 days after the transplantation.

B. Tracing of the swimming path of a representative mouse after neural stem/progenitor cell transplantation. The mouse swimming path was captured by CCD camera and analyzed. Total swimming time until reaching to the target platform of the grafted mice was clearly shortened after 22 days. The improvement has become more evident 45 days after the transplantation.

Carrying the epsilon4 allele of the apoE4 gene was a strong risk factor of AD for humans [57], and apoE4 directly impaired GABAergic inhibitory neuron function [58]. GABAergic interneuron progenitors transplanted into the hippocampal hilus were functionally integrated into the host hippocampus and improved learning and memory function in apoE4 knock-in/APP mice [44]. Thus, alteration in the inhibitory/excitatory balance may underlie the symptomatic changes in patients with AD [59].

Several reports supported the concept that reduction of inhibitory GABAergic synapses was associated with the pathogenesis of AD [60]. Nonetheless, we have to be careful to understand the importance of GABA production in patients with AD. Excessive production of GABA by glial cells may have important roles for the development of neuro-inflammation, leading to neuronal cell death [61,62]. We, and others, focused on GABA-producing neurons and GABAR-expressing neurons. These differences may contribute to differences in the role of GABA in the pathogenesis of patients with AD.

We observed that the majority of VGAT-expressing cells were located around the grafted area in the hippocampus, where defective GABAergic neuronal functions were reported in the dementia model mice [63-65]. With our transplantation protocol, in order to aid re-connection with a shorter distance by axons of the graft between CA1/CA3 and DG of host, we put the cells at the hilus of the DG of the bilateral hippocampi [66]. Thereafter, we found that VGAT+ and GABAR+ neurons were distributed in the hippocampus, especially in the hilus of the DG (Figure 2,3).

VGAT expressing cells composed 10% of the nucleated cells in the hippocampus, and more than 30% of the VGAT positive neurons were human neurons in the hippocampus of the grafted mice. GABAAR+ neurons composed 2.3% of the nucleated cells in hippocampus. In the hippocampus, more than 80% of GABAR expressing neurons were mouse cells.

Taking into account of the fact that the grafts were persistently located near the hilus of the DG, possible mechanisms of restoration of hippocampal cognitive functions by neuronal cell transplantation included:

#1 VGAT+ cells extend their axons both to the pyramidal cell layer (or at least molecular layer) and the granule cell layer to bring about re-connection of their neuronal pathways (Figure 5C) [67, unpublished observation].

#2 Synaptic spillover of GABA may act on GABAR expressing cells and inhibit protein-mediated apoptotic neuronal cell death (Figure 5D) [54,68-71].

Indeed, our preliminary experiments suggested the re-connection by the grafted VGAT positive cells with cells in the granule cell layer and cells in the pyramidal cell layer occurs either directly or indirectly [72]. Thus, phasic inhibition of the connection among grafts and host neurons may play a crucial role in the behavioral improvement of neuron transplanted PDAPP mice. This hypothesis is consistent

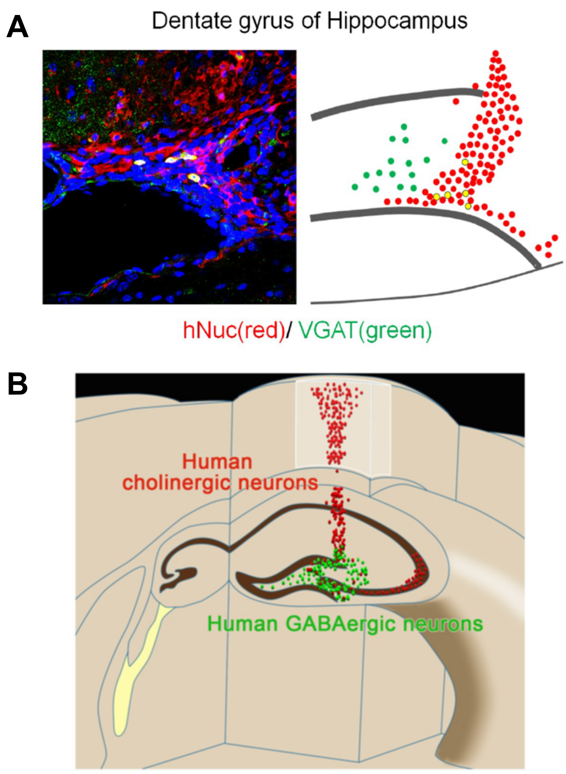

Figure 2: Distribution of human neurons after transplantation of hiPS cell-derived neuronal cells in the AD model mice. The neuronal cells were initially grafted at the hilus of the DG in the mice. A couple of months later, the grafts migrated and were distributed as shown below.

A. Immunohistochemical staining of the hippocampus grafted with hiPS cell-derived neurons. Human cells were detected by anti-human nuclear protein antibody (hNuc; red). GABA producing cells were detected by anti-VGAT antibody (green). The cells were counterstained with DAPI (blue).

B. Distribution of hiPS cell-derived neurons after transplantation. Human cholinergic neurons (red) located throughout the cortex and the CA3 and CA1 of hippocampus. GABAergic neurons (green) located predominantly within the hippocampus, especially near the interface of the DG and CA3. The distribution of both cell types was clearly different from each other after the transplantation. A white rectangle in the cortex indicates the proposed area of the posterior parietal cortex, which plays a role in spatial navigation in rodents [84].

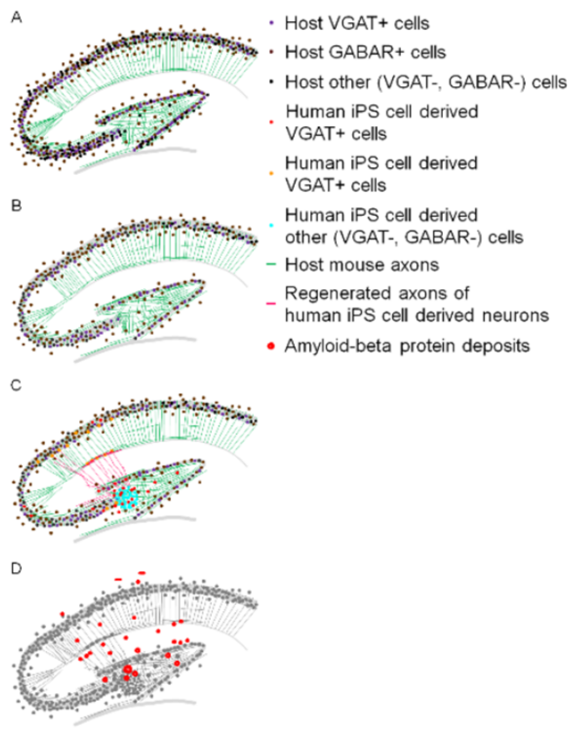

Figure 3: Possible mechanisms of GABAergic inhibitory neuron-induced beneficial effects on neuronal networks of the hippocampus in dementia model mice.

The grafts were initially put on hilus of the DG.

A. A schematic representation of VGAT/GABAR-expressing host cells in the hippocampus of normal mice.

B. A schematic representation of VGAT/GABAR-expressing host cells in the hippocampus of aged PDAPP mice. VGAT+ and GABAR+ cells decreased in number, especially in CA1 and DG subfields.

C. A schematic representation of host and human VGAT/GABAR-expressing cells in the hippocampus of neuron-transplanted PDAPP mice. VGAT+ cells extend their axons both to the pyramidal cell layer (or at least the molecular layer) and the granule cell layer, to bring about re-connection of their neuronal pathways [Suzuki et al., unpublished observation].

D. A schematic representation of amyloid-beta protein deposits in the hippocampus of PDAPP mice. Synaptic spillover of GABA may act on GABAR-expressing cells and inhibit protein-mediated apoptotic neuronal cell death.

with the data of an association between the long-term potential impairment and increased tonic inhibition of GABA in hippocampal neurons of AD model mice [62,73-76]. Possible histological restoration by hiPS cell-derived neuronal cell transplantation into the PDAPP mouse hippocampus is shown in Figure 3B and 4, where inhibitory output provided by the hiPS derived GABAergic neurons may restore the alterations in the inhibitory/excitatory balance.

We are currently investigating the possibility shown in Figure 5, where Gutierrez suggested a possible role of CA3 interneurons in the granule cell-CA3 pyramidal cell connection [77].

In panel A, granule cells of the DG excite pyramidal cells, through giant boutons. The granule cells excite CA3 interneurons to release GABA, inhibit pyramidal cells, and sustain feed-forward inhibition, through boutons en passant and filopodial extensions.

In panel C, an inhibitory response in pyramidal cells to mossy fiber stimulation is due to the activation of interneurons.

We agree with his proposal and taking his proposal into account, we think that our VGAT+ cells substituted the role of CA3 interneurons lost possibly by apoptosis in dementia mice (neurons colored in green in panels B and D). Our preliminary observation suggested that hiPS derived VGAT+ neurons acted on pyramidal cells located in the CA1 and CA3 (panels B and/or D).

Thus, it is possible that our neuronal cell transplantation, which supplemented GABA+/ GABAAR+ cells in the hippocampus, restored impaired GABA/GABAAR circuits in the hippocampus of the PDAPP mice, leading to the restoration of their defective cognitive functions.

In support of our findings, the importance of GABA/ GABAAR circuits was also revealed by administrations of GABA/ GABAAR modulators. NMZ, a positive allosteric modulator of GABAA function, which potentiates the function of the inhibitory neurotransmitter GABA in the brain [77], attenuated the glutamate-induced excitotoxic cascade leading to the inhibition of mitochondrial damage and neuronal loss [78-80].

Selective pharmacological activation of GABAA receptors has been shown to provide neuroprotection against amyloid-beta mediated

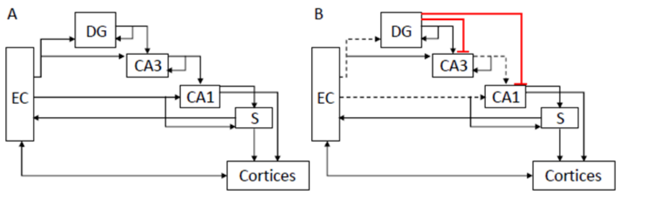

Figure 4: Possible neuronal re-connection by hiPS-derived neuronal transplantation (A part of the figure in "The synaptic organization of the Brain, ED. Gordon M, Shepherd, Oxford University Press (2003)" is modified).

A. Unique unidirectional progression of excitatory pathways (arrows) links each region in the hippocampal formation of normal mice.

B. Several neural pathways are suggested to be preferentially affected in the hippocampal formation of AD model mice (dashed lines) [85]. Red lines indicate possible inhibitory output by the VGAT+ hiPS cell-derived neurons.

EC, Entorhinal Cortex; DG, Dentate Gyrus; CA, Cornu Ammoni

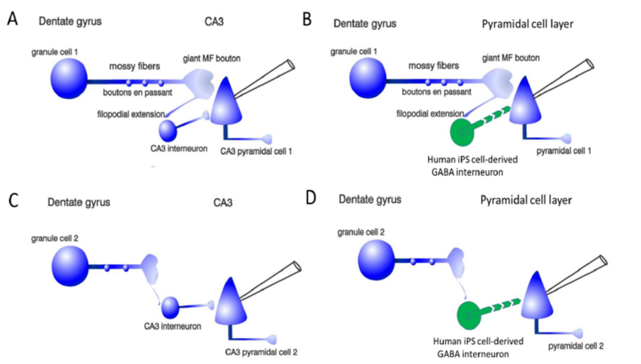

Figure 5: Possible role of hiPS cell-derived GABAergic neurons in the grafted dementia model mice. Activation signals mediated by glutamatergic neurons may be controlled by the hiPS derived neurons (shown in green), thus, they may substitute for the CA3 interneurons lost in dementia model mice (Parts of the figure in [77] are modified).

A. The granule cells of the DG excite pyramidal cells, through giant MF (mossy fiber) boutons. The granule cells excite CA3 interneurons to release GABA, inhibit CA3 pyramidal cells, and sustain feed-forward inhibition, through boutons en passant and filopodial extensions.

B. The granule cells of the DG excite pyramidal cells through giant MF boutons. The granule cells excite the hiPS cell-derived GABA interneuron to release GABA, inhibit pyramidal cells, and sustain feed-forward inhibition, through boutons en passant and filopodial extensions.

C. An inhibitory response in CA3 pyramidal cells after mossy fiber stimulation due to activation of CA3 interneurons.

D. An inhibitory response in pyramidal cells after mossy fiber stimulation due to activation of hiPS cell-derived GABA interneurons.

toxicity, likely through the arrangement of the protein cleavage process [69]. In vitro, chronic activation of GABAA receptor agonists protected cultured neurons against the neurotoxicity of amyloid-beta [81]. However, treatment with picrotoxin, a GABAAR antagonist, also improved the cognitive functions of adult APP/PS1 mice [82].

These findings suggested that phasic and synaptic signals of substances with precise recognition of the receptors' subunits were important for the improvement of AD memory loss.

However, glial production of GABA, possibly by inflammatory responses, may have other implications for the AD pathogenesis [83]. Further studies are needed to clarify this.

Thus, the interaction between GABA, secreted predominantly by the grafted neurons, and receptor (GABAR) expressing grafted neurons and host neurons, may underlie the improvement of memory performance in the PDAPP mice that have undergone transplantation.

Conclusions

Transplantation of hiPS cell-derived neurons is a promising candidate for the treatment of advanced AD. The graft's autonomous effects on the regeneration of damaged neuronal circuits, possibly involving ACh and GABA are attractive mechanisms for clinical application. Further studies are needed to confirm the roles of ChAT-positive cells and VGAT-positive cells in functional recovery before conducting clinical application in patients with AD.

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